REMARKS

The Office Action, dated August 27, 2003, objected to claims 7-10 and rejected claims 7, 8 and 9. Claim 10 was not treated on the merits. The claims were also subject to a telephonic restriction requirement. Applicant herein affirms election of claims 7-10, corresponding to the invention of Group II.

Upon entry of the present amendment, claims 1-10 are pending. In the present amendment, Applicant has amended claims 7-10 to further clarify the invention. The amendments are fully supported by the specification and the originally filed claims, as summarized in the table below. No new matter is added by these amendments.

Claim	Phrase	Examples of Support in the Specification/Original Claims
7, 8, 10	having activity greater than or equal to an original enzyme protein	original claim 7, part (4); original claim 8, part (4); original claim 10, part (4)
7, 8	with codons encoding	page 6, line 21
7, 8	to obtain a mutant enzyme protein	page 6, lines 30-31; page 7, line 28
7	enzyme protein having a substitution A1/MA1	page 6, line 26
7, 8	encoding any of L-alanine...L-tryptophan	original claim 1
7,8	for a maximum of 18 different substitutions at any Ai	page 6, lines 27-30; page 7, lines 29-32
7	a maximum of three mutant enzyme proteins	original claim 7, part (3); page 7, lines 12-13
7	thereby obtaining mutant enzyme proteins having substitutions Ai/Bi1, A1/Bi2, and Ai/Bi3	page 7, lines 12-13
7	repeating (2) for all Ai	page 7, line 14

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7	producing a sulfur atom-free enzyme protein comprising any combination of the substitutions in (2) and (3) with the substitution of A1/MA1 in (1), wherein a substitution occurs at all Ai	page 7, lines 14-16
7, 8	selecting a sulfur atom-free enzyme protein	page 7, lines 16-17
8	selecting a maximum of three double mutants	page 7, lines 35-36
8	repeating the stepwise substitution of codons encoding sulfur atom-containing amino acids at each remaining Ai (i = 4 to n) in the same manner as in (2) and (3) wherein positions Ai-An are substituted in any order	page 8, lines 7-12
	measuring enzyme activity of sulfur atom-free enzyme proteins obtained upon substitution of An	page 8, lines 7-9
9	wherein A1-An are substituted in order of position on the amino acid sequence	original claim 8
10	using a combination of a combined mutation method and a stepwise mutation method	page 9, lines 33-34
10	[parts (1)-(5)]	original claims 7 and 9; page 8, lines 18-35; page 9, line 30 - page 11, line 1 (see also support for amendments to claims 7 and 8 above)

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I. Objections to Claims 7-10 Under 37 CFR 1.75(c)

The Examiner objected to claim 10 under 37 C.F.R. 1.75(c) as being in improper multiple dependent form. Office Action at page 3. Claim 10 has been amended to depend from claim 8 only. The Examiner also objected to claims 7-9 as depending from

non-elected claim 1. *Id.* Claims 7-9, as amended, no longer depend directly or indirectly from claim 1. The Examiner further objected to claims 7-8 because of a number of informalities and suggested ways to correct those informalities. *Id.* The Applicant has amended the claims in accordance with the Examiner's suggestions or in a manner that otherwise overcomes the Examiner's objections. In view of the foregoing amendments, withdrawal of the objections to claims 7-10 is respectfully requested.

II. Rejection of Claims 7-9 Under 35 U.S.C. § 112, 2nd paragraph

The Examiner rejected claims 7-9 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite. Specifically, the Examiner stated that "[c]laims 7 and 8 are confusing in the recitation of 'L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline codon' as **a codon** is a sequence encoding a single amino acid." Office Action at page 4. Applicant has amended claims 7 and 8 to recite "with **codons** encoding any of L-methionine-L-alanine, L-methionine-L-serine, or L-methionine-L-proline."

The Examiner also rejected claim 7, part (2), as allegedly confusing in the recitation of "codons encoding sulfur-containing amino acids at another sites Ai (i = 2 to n) are substituted with codons encoding another another amino acids among the 18 types of amino acid according to claim 1." *Id.* Applicant has amended claim 7, part (2), to recite "a codon encoding a sulfur-containing amino acid at a position Ai (i = 2 to n) is substituted with a codon encoding any of L-alanine, L-aspartic acid, L-glutamic acid, L-phenylalanine, L-glycine, L-histidine, L-isoleucine, L-lysine, L-leucine, L-asparagine, L-proline, L-glutamine, L-arginine, L-serine, L-threonine, L-valine, L-tyrosine, and L-trypotophan . . . ."

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The Examiner further rejected claim 8, part (4), as allegedly confusing in the recitation of "preparing a quadruple mutant, ...,". *Id.* Applicant has amended claim 8, part (4), to recite "repeating the stepwise substitution of codons encoding sulfur-containing amino acids at each remaining Ai (i = 4 to n) in the same manner as in (2) and (3), wherein positions Ai-An are substituted in any order . . . ."

The Examiner further rejected claim 9 as allegedly confusing in the recitation of "according to any one of (n! types) permutations and combinations of A1, A2, ... An." *Id.* Applicant has amended claim 9 to recite "wherein A1-An are substituted in order of position on the amino acid sequence," and claim 8, part (4), to recite "wherein positions Ai-An are substituted in any order."

In view of the foregoing amendments, claims 7-9 are clear and definite. Withdrawal of the rejection of these claims under 35 U.S.C. § 112, second paragraph, is respectfully requested.

III. Rejection of Claims 7-9 Under 35 U.S.C. 112, 1st paragraph

The Examiner rejected claims 7-9 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement. Specifically, the Examiner stated that "the specification, while being enabling for methods of construction [of] the sulfur-free DHFR genes of Table 7 of the specification and a sulfur-free xylanase gene encoding SEQ ID NO:9, does not reasonably provide enablement for methods of constructing any sulfur-free enzyme having activity greater than or equal to the activity of the original protein." Office Action at page 5. Because the Examiner has applied an incorrect standard of enablement, this rejection is respectfully traversed.

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The Examiner's rejection is based on the assertion that "the specification does not establish (A) which sulfur containing proteins have [sic] may be modified without effecting activity; (B) the general tolerance of enzymes to modification and extent of such tolerance in particular with respect to the modification of cysteine residues . . . ; and (C) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful." Office Action at page 7.

The Applicant is aware that substitution of certain amino acids, particularly cysteine, may result in an inactive protein or a protein with reduced activity. Such proteins, however, fall outside the scope of the claims. The claims recite, for example, ". . . selecting a sulfur atom-free enzyme protein having activity greater than or equivalent to that of the original enzyme protein . . . ." (See, e.g., claim 7, part (4).) Therefore, the claims expressly exclude inoperative embodiments, such as methods that produce inactive or less active proteins, because the claimed methods do not select for such proteins.

Furthermore, the specification fully enables the claimed methods of producing a sulfur atom-free enzyme protein having activity greater than or equivalent to an original enzyme protein. To enable the claims, the Examiner appears to require that Applicant test all proteins for tolerance of substitutions at methionine and in particular, cysteine. The test for enablement imposes no such requirement. The correct test of enablement is whether one reasonably skilled in the art could make or use the claimed invention without undue experimentation. *See* M.P.E.P § 2164.01 at 2100-178 – 2100-179 (8th ed., rev. 1 (2003). When inoperative embodiments are present, the test involves whether one skilled in the art could determine operative embodiments without undue

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experimentation. *See id.* § 2164.08(b) at 2100-192 – 2100-193. Furthermore, "a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed . . . ." *In re Wands*, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988).

The present claims are directed to methods of producing sulfur atom-free enzyme proteins. One skilled in the art can carry out the claimed methods on any protein of interest without undue experimentation. In a method of the invention, for example, a methionine or cysteine in an enzyme protein is substituted by any of the sulfur-free amino acids, thus generating mutant enzyme proteins. The activity of the mutant enzyme proteins is measured. A maximum of three mutant proteins with the highest activity are selected. Further substitution at another methionine or cysteine takes place, and selection is repeated. Although the method could involve a number of rounds of mutagenesis and selection, such experimentation is nonetheless reasonable, because the method provides a routine and systematic way of arriving at substitution mutants that have the desired activity.

The Examiner argues that the result of multiple amino acid substititutions within a protein is not predictable with respect to the protein's activity. Office Action at page 6. This assertion, however, is not relevant, in view of the subject matter that is claimed. The test for enablement here is whether one skilled in the art, in following the claimed method, can determine which proteins have the desired activity without undue experimentation, *not* whether the result of multiple amino acid substitutions is predictable. *See* M.P.E.P § 2164.01 at 2100-178 – 2100-180 (stating that it is improper

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to conclude that a disclosure is nonenabling based solely on analysis of a single *Wands* factor). Moreover, the Examiner should focus on the claimed method as a whole, and not just on a particular aspect of the method. *See id.* at 2100-191 (In analyzing questions of enablement, "[t]he examiner should determine what each claim recites and what the subject matter is when the claim is considered as a whole, not when its parts are analyzed individually.") (emphasis added and in original).

The Examiner also incorrectly contends that "the disclosure is limited to the construction of very few sulfur-free enzymes from only 2 specific parent genes, both of which in fact naturally were free of cysteine residues." Office Action at page 6. In fact, the starting protein for the "ANLYF" sulfur atom-free mutant of dihydrofolate reductase (DHFR) was a cysteine-free mutant designated AS-DHFR. See specification, paragraph bridging pages 8-9. In AS-DHFR, two cysteine residues are substituted by alanine and serine, respectively, relative to the wild type enzyme. (Compare residues 85 and 152 of accession no. P00379, enclosed for the Examiner's convenience, with residues 85 and 152 of SEQ ID NO:1.) Further substitution of all methionine residues according to the claimed method resulted in the "ANLYF" sulfur atom-free mutant, as well as eight other sulfur atom-free mutants, having activity greater than the wild type DHFR. See specification at page 10, last full paragraph, and paragraph bridging pages 10-11. Thus, the specification provides a sulfur atom-free enzyme of the desired activity that lacks cysteine and methionine residues otherwise present in the wild type enzyme and that was obtained without undue experimentation.

Applicant also points out that it is not necessary for one skilled in the art to know which proteins in the universe of proteins may undergo substitution without losing

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activity and which proteins in particular are tolerant to cysteine substitution in order to practice the claimed methods. One skilled in the art only needs to know whether his or her protein of interest is tolerant of substitution. As stated above, one skilled in the art can make this determination using the routine methods set forth in the claims.

In view of the foregoing remarks, Applicant submits that the specification fully enables the claims. Withdrawal of the rejection of claims 7-9 is respectfully requested.

IV. Rejection of Claims 7-9 Under 35 U.S.C. § 103(a)

The Examiner rejected the claims under 35 U.S.C. § 103(a) as allegedly being unpatentable over the combined disclosures of Li et al. (1995) *Biotech. and Bioeng.* 48:490-500, and Recktenwald et al. (1993) *J. Biotech.* 28:1-23, in view of Lathrop et al. (1992) *Prot. Exp. and Pur.* 3:512-17, and Barnett et al., WO 96/30481. Because the cited references, either singly or in combination, fail to teach or suggest the claimed methods, this rejection is respectfully traversed.

The Examiner stated that "[i]n view of the combined disclosures of Li et al. and Recktenwald et al. the ordinary skilled artisan would have found it obvious to use site specific mutagenesis to replace all the methionine and cysteine residues of any enzyme which is used in oxidative conditions with more oxidatively stable amino acids . . . ." Office Action at page 10. Applicant respectfully contends that the combined disclosures of Li and Recktenwald do not teach or suggest replacing all methionine and cysteine residues. Li generally discusses that methionine, cysteine, histidine, tryptophan, and tyrosine "are most susceptible to oxidative modifications." (See abstract and page 491, col. 2.) Li also discusses substitution of a single reactive methionine residue of α_1-antitrypsin inhibitor (see page 497, col. 1). Recktenwald generally lists cysteine,

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methionine, and tryptophan as targets for protein engineering. (See page 3, Table 2.) Recktenwald also refers to a publication by Estell et al., wherein a single methionine residue was substituted. (See abstract of Estell et al. (1985) *J. Biol. Chem.* 260:6518-21, enclosed for the Examiner's convenience.) Neither reference specifically discusses substituting all methionine and cysteine residues in a single protein.

The decision to focus on all methionine and cysteine residues (with or without selection of other oxidatively susceptible amino acids) as targets for substitution is a specific choice provided solely by the Applicant's specification. The Examiner's argument is thus based on hindsight reconstruction, or at best, an "obvious to try" rationale, wherein "what would have been 'obvious to try' would have been to . . . try each of numerous possible choices until one possibly arrived at a successful result." *See* M.P.E.P § 2145(X.)(B.) at 2100-156; *see also In re Geiger*, 2 USPQ2d 1276, 1278 (Fed. Cir. 1987) ("At best, in view of these disclosures, one skilled in the art might find it obvious to try various combinations of these known scale and corrosion prevention agents. However, this is not the standard of 35 U.S.C. § 103(a).") Here, the art generally discusses substitution of oxidation-susceptible amino acids including methionine, cysteine, tryptophan, tyrosine, and histidine. This does not suggest substituting all methionine and cysteine residues. Thus, the combination of Li and Recktenwald fails to teach or suggest the claimed methods.

As the combination of Li and Recktenwald fails to teach or suggest substituting all methionine and cysteine residues, there is no motivation to use the methods set forth in Barnett and Lathrop. Although Barnett discusses making multiple methionine substitutions (excluding the initiator methionine), it does not discuss substituting all

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methionine residues. Although Lathrop discusses substituting the amino acid following the initiator methionine to allow removal of the initiator methionine by aminopeptidase, it also does not discuss substituting all methionine residues. As discussed above, the art provides no motivation to combine these references. Therefore, Li and Recktenwald fail to render the claims obvious in view of Barnett and Lathrop.

Applicant further notes that the Examiner's rejections under both 35 U.S.C. § 112, first paragraph, and 35 U.S.C. § 103(a) are contradictory. In making these rejections, the Examiner is asserting that the specification does not enable one skilled in the art to practice the claimed methods, yet at the same time, the Examiner is also asserting that one skilled in the art can arrive at the claimed methods with a reasonable expectation of success based on the combination of the cited references. How can the specification, combined with the knowledge of those skilled in the art, fail to enable the claims, if at the same time, the level of skill in the art allows the skilled artisan to practice the claimed invention with a reasonable expectation of success? The Examiner has taken two contradictory positions.

In view of the foregoing remarks, Applicant requests withdrawal of the rejection of claims 7-9 as being obvious under 35 U.S.C. 103(a).

CONCLUSION

In view of the foregoing amendments and remarks, Applicant respectfully requests reconsideration and reexamination of this application and the timely allowance of the pending claims. If the Examiner does not consider the application to be

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allowable, the undersigned requests that, prior to taking action, the Examiner call her at (650) 849-6778 to set up an interview.

Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: January 27, 2004

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